# growing interest in outsourcing ION CHANNEL services

Significant demand now exists in Pharma and Biotech to access ion channel testing services and this is reflected by the number and the variety of service offerings. The majority of GLP cardiac safety hERG testing is outsourced today using manual patch clamping. Automated patch clamping (APC) systems are, however, undertaking an ever increasing role in the services offered and there appears to be increasing acceptance of their use as alternatives to the 'gold standard' manual patch clamp. This reflects recent improvements in quality of data generated with APC systems and the significant wealth of experience gained in their use. In the highly competitive area of cardiac safety hERG testing price and turn-around time are now key factors in the selection of a fee-for-service provider. CROs are also competing to offer new services and some are assembling large ion channel libraries, variously grouped into panels for use in selectivity profiling where ion channels are the primary drug targets or are off-targets for safety and toxicology. Other services on the increase include the capability to accept larger libraries for primary screening, various cardiotoxicity assessment offerings and ion channel directed chemical libraries. For the future screening services involving primary cells, cardiomyocytes and stem cells seem likely.

**By Dr John Comley**

Ion channels play key roles in regulating cardiac, neuronal and secretory tissues function and effective modulation of ion channel activities impacts on many disease states. Ion channels rank among the most studied target classes (with kinases and GPCRs) and their investigation is a high priority in many organisations. However, the direct measurement of ion channel function has until recently been a difficult task to accomplish and the lack of suitable technologies applicable to high-throughput screening (HTS) has hindered ion channel exploitation compared to other target classes. For this reason the cost of an ion channel data point (well screened) was one of the most expensive to generate within drug discovery. Recent advances in automated patch clamping (APC) have, however, ensured that high quality meaningful safety pharmacology data can now be generated early in drug discovery and at higher throughput than conventional manual patch

**Figure 1: Ion channel drug discovery – technology preference**

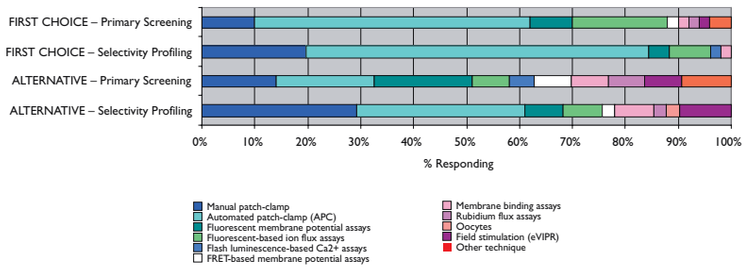


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**Figure 2: Ion channel drug discovery – interest in outsourcing**

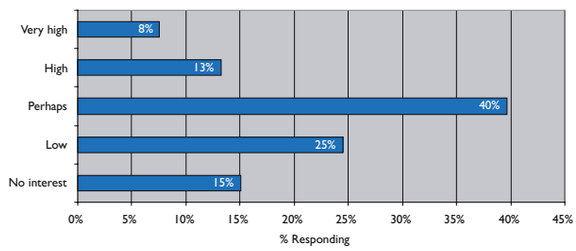


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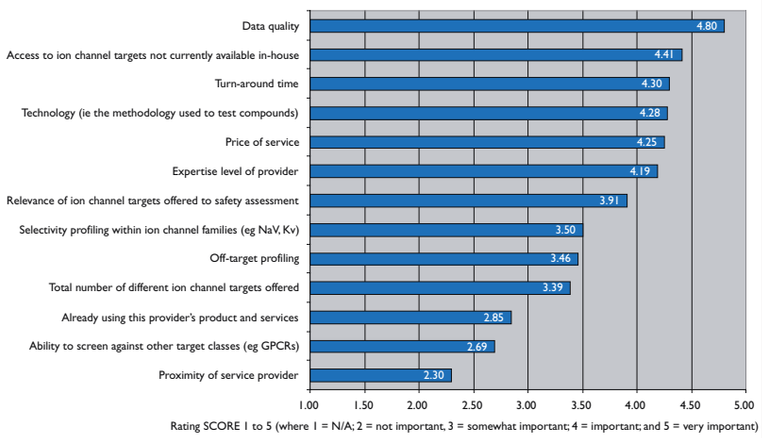
**Figure 3: Ion channel drug discovery – most important factors in choosing a fee-for-service provider**

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clamping. One of the most popular applications of APC systems is the assessment of QT prolongation as a result of inhibition of the hERG potassium channel. Interest in this area is growing for two reasons; firstly, a number of drugs have been withdrawn from late stage clinical trials due to these cardiotoxic effects; and secondly, it is now a recommendation to test for this liability under the ICH regulatory guidelines (S7B). Owing to the highly specialised nature of ion channel assays and mandatory safety requirements for IND submissions, a significant number of fee-for-service providers have set up operations to address the growing outsourcing needs of ion channel research and off-target safety concerns. Services offered range from fully GLP-compliant cardiac safety hERG testing, early non-GLP hERG safety testing, ion channel selectivity profiling, primary screening and *in vitro* cardiotoxicity assessment. HTStec's 4th Annual Ion Channel Trends 2007 survey and report[1] in addition to monitoring ion channel screening metrics, had a focus on the needs and requirements for outsourced ion channel services. In this article we review some of the reports findings on outsourcing together with the ion channel services offerings of a number of fee-for-service providers.

## Ion channel drug discovery services

It is perhaps not surprising, with recent APC product developments and user focus on direct measures of ion channel activity, that the first choice assay technology survey respondents were most interested in accessing at a fee-for-service providers for ion channel drug discovery (ie primary screening and selectivity profiling) was automated patch clamping. Acceptable alternative technologies for primary screening were fluorescent membrane potential assays, automated patch clamping and manual patch clamp. Acceptable alternative technologies for selectivity profiling were automated patch clamping and manual patch clamp (**Figure 1**).

The majority (65%) of current interest in outsourcing ion channel screening for drug discovery was rated between 'low' and 'perhaps'. Only 21% of survey respondents had either a 'high' or 'very high' current interest in outsourcing ion channel screening for drug discovery (**Figure 2**).

Data quality was the main factor cited by survey respondents when choosing a fee-for-service provider for screening ion channels for drug discovery. This was closely followed by access to ion channel targets not currently available in-house; technology (ie the methodology used to test compounds) and then turnaround time (**Figure 3**).

## Cardiac safety hERG testing

APC systems were preferred by survey respondents for non-GLP cardiac safety hERG testing (with

greatest interest in the MDC Ionworks Quattro/HT and MDC PatchXpress systems) and manual patch clamp for GLP cardiac safety hERG testing (**Figure 4**).

The majority of survey respondents undertake non-GLP cardiac safety hERG testing primarily in house, while GLP cardiac safety hERG testing was primarily outsourced (**Figure 5**).

41% of survey respondents rated their current interest in outsourcing cardiac safety hERG testing as either 'high' or 'very high'. A further 38% of survey respondents rated their current interest in outsourcing cardiac liability hERG testing as 'perhaps' (**Figure 6**).

Price of service and turn-around time were the main factors cited by survey respondents when choosing a fee-for-service provider for cardiac liability testing (**Figure 7**).

## *In vitro* cardiotoxicity assessment

Cardiotoxicity assessment is usually taken to refer to the more direct measurement of delayed repolarisation, eg prolongation of action potential duration at 90% repolarisation (APD90) or QT interval. The hERG assay is a subset of cardiotoxicity assessment with hERG testing as a marker for delayed repolarisation and QT prolongation (the S7B guidance). Many fee-for-service providers advocate a cardiac channel panel as the most complete assay for *in vitro* cardiac risk assessment.

The majority of survey respondents are making greatest use of automated hERG patch clamp and manual hERG patch clamp systems to undertake *in vitro* cardiotoxicity assessment. Other systems, eg hERG receptor binding methods and Purkinje fibre analysis were less widely used to undertake *in vitro* cardiotoxicity assessment. The Purkinje fibre assay assesses multi-channel effects and is suggested in S7B guidance (**Figure 8**).

The majority (57%) of survey respondents rate the value of using *in vitro* cardiotoxicity assessment for safety evaluation as important (ie one of the main factors considered). A further 29% of respondents rated it as extremely important (ie will drive the key decision) (**Figure 9**).

Combined 74% of survey respondents expect their usage of outsourced *in vitro* cardiotoxicity assessment to increase over the next 12 months, with a calculated mean +6% change of usage of outsourced *in vitro* cardiotoxicity assessment expected over the next 12 months (**Figure 10**).

The inadequate decision-making power of the results was selected by the majority (49%) of survey respondents as the most significant challenge to *in vitro* cardiotoxicity assessment today. In

**Figure 4: Cardiac safety hERG testing – technology preference**

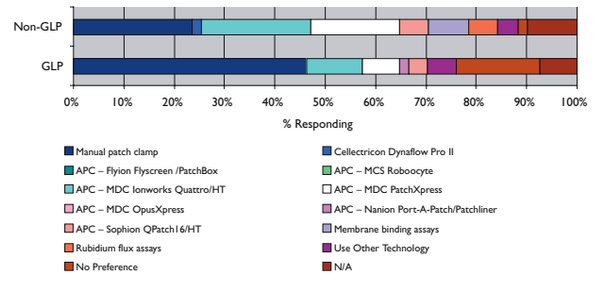





**Figure 5: Cardiac safety hERG testing – preferred approach**

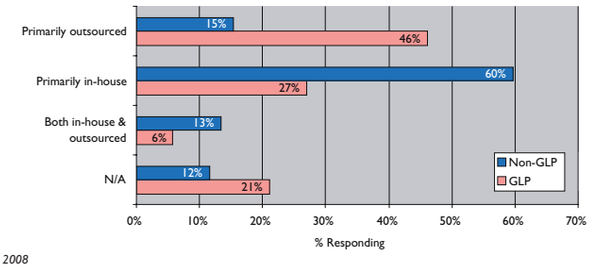





**Figure 6: Cardiac safety hERG testing – interest in outsourcing**

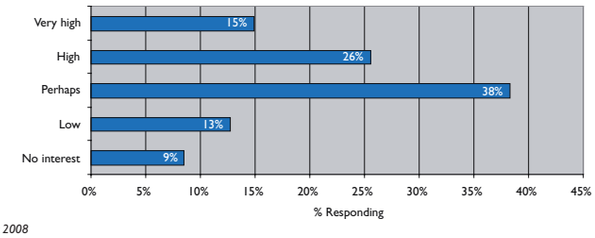





**Figure 7: Cardiac safety hERG Testing – most important factors in choosing a fee-for-service provider**

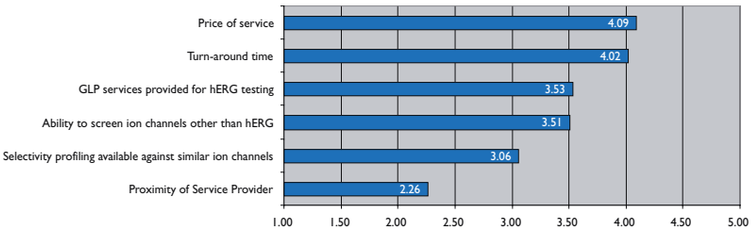


**Figure 8: *In vitro* cardiotoxicity assessment – technology approach**

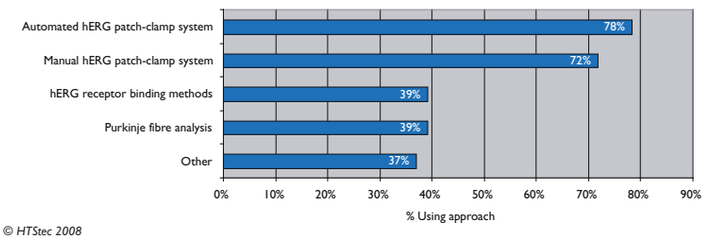





**Figure 9: *In vitro* cardiotoxicity assessment – importance in safety evaluation**

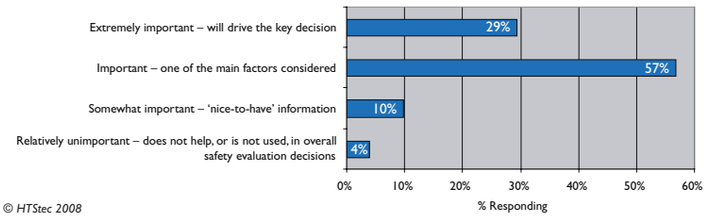





**Figure 10: Outsourced *in vitro* cardiotoxicity assessment – expected usage over the next 12 months**

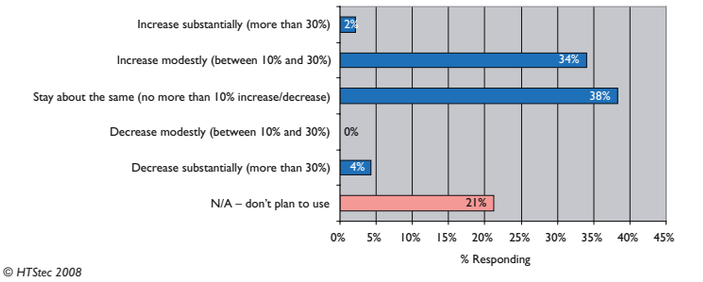
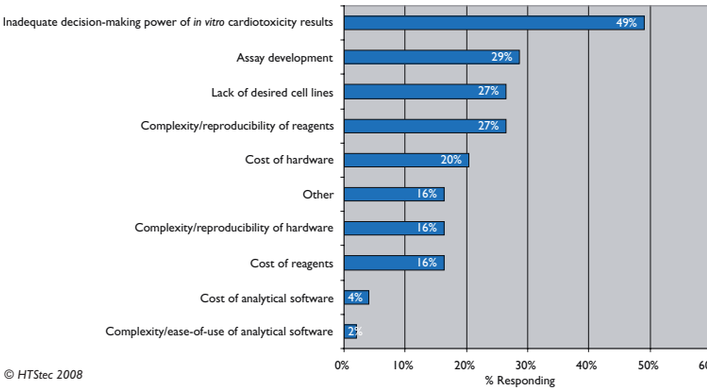





**Figure 11: *In vitro* cardiotoxicity assessment – most significant challenges**






comparison other aspects rated were considered much less challenging (**Figure 11**).

## Fee-for-service providers of ion channel services

Survey respondents clearly ranked ChanTest the most trusted fee-for-service provider of outsourced ion channel services, it was followed by Cerep, MDS Pharma Service and then Millipore (**Figure 12**).

**Table 1** summarises the range of ion channel services offered by service providers in this area. None apart from ChanTest currently attempts to address the full range of services outlined in the table.

The following are vendor-contributed updates that highlight some of the details of each vendors current and planned offering.

**Aurora Biomed**'s (www.aurorabiomed.com) contract research service offerings have historically been focused on its proprietary ion flux-based screening services for HTS of ion channel targets. However, it is now offering a broader range of services to cater for contract research in other areas of drug discovery, including target validation and cardiac safety assessment. Aurora Biomed offers electrophysiology (EP) services for a variety of ion channel (TRP, $Kv_{1.3}$, $Nav_{1.5}$, etc) and neurotransmitter receptor (NMDA, AMPA, and mGLUs) targets. For cardiac safety assessment, a panel of cardiac ion channels is available: $Nav_{1.5}$ ($I_{Na}$), $Kv_{4.3}$ ($I_{to}$), $Kv_{1.5}$ ($I_{Kur}$), $Cav_{1.2}$ ($I_{ca}$), KvLQT1-mink ($I_{Ks}$), hERG ($I_{Kr}$), $Kir_{2.1}$ and $Cav_{3.2}$. EP screens are carried out on stably or transiently expressed targets in *Xenopus* oocytes, and CHO or HEK cell lines. Depending on the requirements of the client, services can include dose-response curve determination, compound effects on I-V and G-V curves, and kinetic studies on voltage-independent inactivation. In addition, the *Xenopus* oocyte platform can be used to characterise ion channel cDNA mutants and transiently express ion channel cDNA at appreciable throughput. For clients with higher throughput needs, Aurora Biomed continues to offer ion flux-based HTS platforms for lead identification and lead optimisation. In addition to ion channel screening in stably expressed cell lines the following are also now available: 1) screening of α1, α2, and α3 $Na^+/K^+$-ATPase isoforms in endogenous cells; 2) screening of mouse cardiomyocytes (for various ion channels); and 3) hybrid lipid vesicle screening (for various ion channels) (**Figure 13**).

**AVIVA Biosciences** (www.aviva.com) innovated high resistance voltage clamp chips, presently used in the PatchXpress automation system. AVIVA has provided hERG screening contract service since 2003, and continues to develop new ion channel screening assays based on the PatchXpress automation system, including multiple human-relevant sodium channels and potassium channels. Recently, AVIVA has added calcium channels and various ligand-gated channels to its panel of offerings. In addition to the ongoing developments in the PatchXpress-based products, AVIVA offers conventional electrophysiology services on various cells that do not easily lend themselves to automation, focusing especially on cardiac safety testing. AVIVA is expanding its collaborative efforts with industry and larger scale academic partners interested in library screens against ion channel families. In the coming months, AVIVA will expand its portfolio from cardiac-based services into other human-relevant systems, building on its competence in the ion channel field.

**Figure 12: Most trusted provider of outsourced ion channel services**

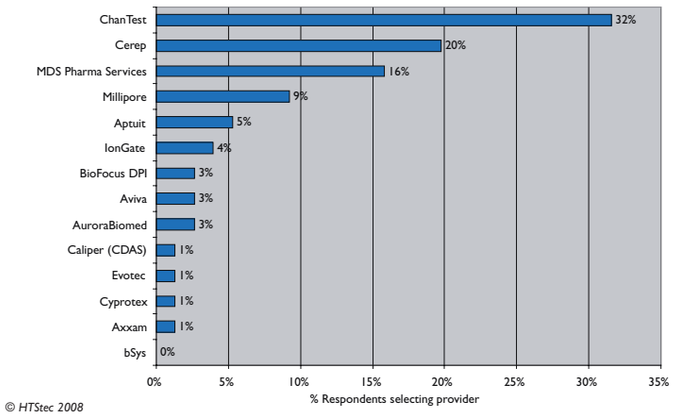


**Figure 13**
HTS services using Aurora Biomed's commercially available Ion Channel Reader ICR 12000 based on atomic absorption spectroscopy

**Axxam** (www.axxam.com) offers functional cell-based HTS assays for ligand/voltage-gated ion channels, electrogenic transporters and ion exchangers. Its flash luminescence Photina® technology allows particularly high sensitivity and its fully automated screening station is able to measure up to 50,000 test-points/day. With appropriate assay design and careful analysis of kinetic responses it can distinguish between agonistic and antagonistic responses, as well as determine if the modulation is allosteric or competitive in a single well. Allosteric modulators are often among the most interesting hits for ion channels, since they are often self-limiting and reversible, unlike some competitive inhibitors. For following up after a primary screen, Axxam uses its existing battery of functional ion channel assays to conduct compound profiling and selectivity test analysis to determine the affinity of identified ligands and

**Table 1:** Summary of ion channel testing offered by service providers. * *Non-APC includes radioligand binding, fluorescence, flash luminescence, oocytes,*

| ION CHANNEL SERVICE PROVIDER | ION CHANNEL DRUG DISCOVERY | | | |
|---|---|---|---|---|
| | Primary Screening | | Selectivity Profiling | |
| | Non-APC* | APC | Non-APC* | APC |
| Aurora Biomed | ✓ | | ✓ | |
| Aviva Biosciences | | ✓ | | ✓ |
| Axxam | ✓ | | ✓ | |
| BioFocus DPI | ✓ | ✓ | ✓ | ✓ |
| bSys | ✓ | ✓ | ✓ | ✓ |
| Caliper Discovery Alliances & Services | | | ✓ | |
| Cerep | ✓ | ✓ | ✓ | ✓ |
| ChanTest | ✓ | ✓ | ✓ | ✓ |
| Cyprotex | | | | ✓ |
| Cytocentrics | | | | ✓ |
| Evotec | ✓ | ✓ | ✓ | ✓ |
| MDS Pharma Services | ✓ | | ✓ | ✓ |
| Millipore | | ✓ | | ✓ |

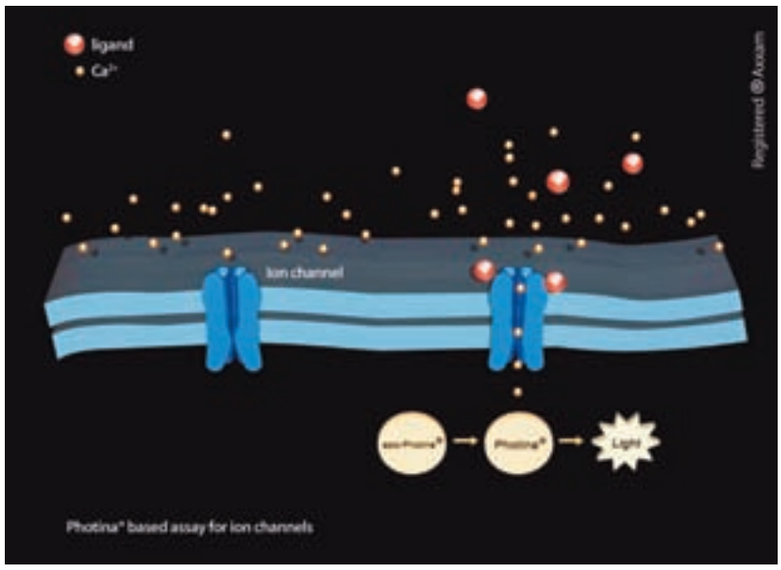



**Figure 14:** Axxam flash luminescence Photina® technology for ion channel HTS

ensure their specificity. Currently, Axxam can design assays using insect cells and primary human cells which endogenously express the ion channels upon *in vitro* differentiation. Future developments envision the use of mouse stem cells differentiated *in vitro* into various cell lineages, such as neurons, for HTS to get as close to the *in vivo* state of the receptors as HTS technology allows (**Figure 14**).

**BioFocus DPI** (www.glpg.com) is a fee-for-service research company offering both biology and medicinal chemistry expertise for early stage collaborative ion channel drug discovery. Biology services include cDNA cloning and cell line generation, assay development, screening services using both

*scence, oocytes, ion flux assays etc*

| | CARDIAC SAFETY HERG TESTING | | IN VITRO CARDIOTOXICITY ASSESSMENT | ION CHANNEL PROFILING LIBRARIES | MEDICINAL CHEMISTRY SERVICES |
|---|---|---|---|---|---|
| | Non-GLP | GLP | | | |
| | ✓ | | ✓ | ✓ | |
| ✓ | ✓ | | Planned | Planned | |
| | ✓ | | | | |
| ✓ | ✓ | | | | ✓ |
| ✓ | ✓ | ✓ | ✓ | | |
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**Figure 15:** Ion channel testing using the PatchXpress® at BioFocus DPI

electrophysiology-based methodology and high throughput screening (HTS) techniques based around fluorescence, luminescence and radiometric assay formats. Comprehensive electrophysiology-based screening resources offer a range of throughputs, with two IonWorks® Quattro™ 384 well platforms, PatchXpress® 16 channel giga seal quality automated recording and conventional electrophysiology techniques able to contribute at all stages of a research programme. Recent successful electrophysiology-based screening campaigns have ranged between 4,000 and 70,000 compounds, with particular emphasis placed upon providing IonWorks® Quattro™ voltage protocols that maximise the primary screen information content regarding mechanism of compound activity. This data is particularly important for ion channel targets, where potential binding sites change due to gating transitions and targeting of a particular state of the channel can enhance the therapeutic usefulness of a compound. Additional key strengths of BioFocus DPI include comprehensive in-house medicinal chemistry expertise combined with a 700,000 compound diverse collection and SoftFocus® ion channel directed libraries targeting both voltage- and ligand-gated families. Clients may therefore elect to screen the full diversity compound deck using traditional HTS techniques or, in collaboration with BioFocus DPI chemists, make an intelligence based selection of a smaller set compounds which is particularly suited to 384 well automated electrophysiology-based screening. Recent experience has demonstrated the advantages of this approach which can provide an enriched hit rate and compounds pre-selected for advantageous tractability (**Figure 15**).

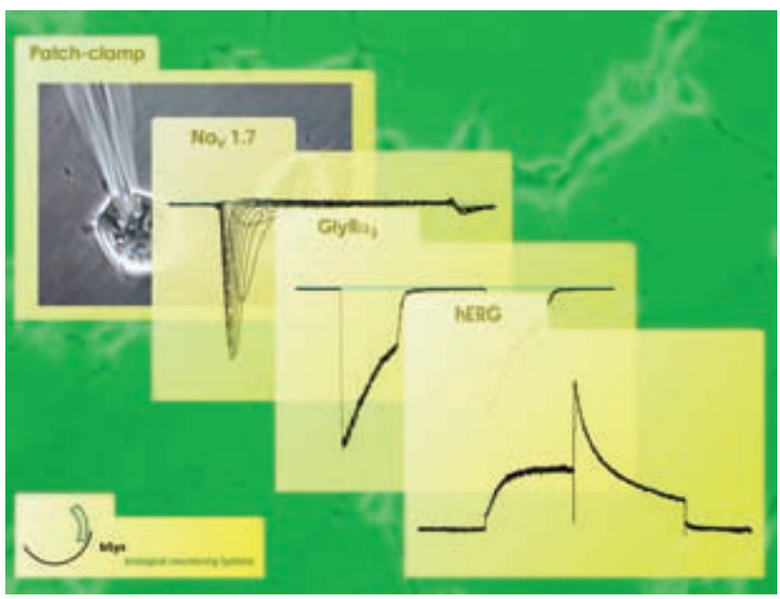


**Figure 16:** In the ion channel screening domain bSys provides high quality electrophysiological data in all relevant categories by rigorous implementation of external and internal standards

**bSys** (www.bsys.ch) is a GLP accredited Swiss CRO focusing on functional membrane protein screening using patch-clamp and fluorescence techniques. GLP quality control systems apply to all assays in both GLP and non-GLP studies. Having acquired substantial expertise with cardiac safety relevant channels (mostly hERG) bSys broadened its library of ion channel assays to CNS (central nervous system) targets, ie pain and epilepsy-relevant ligand-gated and voltage-gated channels, and transporters. With the advent of novel suitable fluorescent dyes, fluorescence assays instead of radioactive assays became state of the art. Services at bSys also include the rapid development of cell lines with high target expression, suitable for fluorescence and patch-clamp assays. The latest release is the first reliable high expression CHO clone (HERGDUO™) co-marketed with Sophion (Denmark). The highest quality in patch-clamp screening is bSys's goal. Unfortunately, not all screening laboratories address this need, especially if automated patch-clamp systems are used at high throughput. Poor quality reduces statistical significance of patch-clamp data, impairs successful lead optimisation and also leads to questionable inter-laboratory or inter-system comparability of results. To provide maximum quality for patch-clamp assays, key parameters are run-down and stability of seal and series resistance has to be controlled (implications for hERG screening is

shown on http://bsys.ch/flyer/quality/flyer.html). In bSys's experience, the strict application of quality criteria does not interfere with throughput and cost-effectiveness. However, it requires continued staff education towards precision, the use of high expression clones and slightly higher expenses for disposables (**Figure 16**).

**Caliper Discovery Alliances & Services** (www.caliperls.com/products/contract-research/) currently offers more than 30 ion channel assays in both binding and cell-based formats representing both ligand- and voltage-gated ion channels. It is noteworthy that in recent years there has been an increased level of concern in safety pharmacology around cardiac repolarisation. Fuelled by this interest, funds from the National Institute of General Medical Sciences (NIGMS) supported the development of its most recent addition of an array of recombinant cell lines expressing nearly all $K^+$-channels involved in cardiac repolarisation, one of which is the well known hERG ($Kv_{11.1}$) potassium channel. Caliper continues to expand its ion channel assays in binding and cell-based formats in order to augment its safety pharmacology service platform; and to comprehensively address the concerns of its broad customer base, including pharmaceutical and biotech companies, as well as academic institutions. Additionally, Caliper currently has a Phase II research project with which it has built a computational model to forecast hERG inhibition. This will prove to be a valuable tool to triage libraries before they are sent to screens.

**Cerep** (www.cerep.com) employs two technologies to assess the hERG liability of a test compound: patch-clamp assay (both automated and conventional patch clamp) and radioligand binding assay. Conventional patch clamp is offered in both GLP-compliant and non-GLP formats. In hERG binding assay, test compounds are studied for their ability to displace the radiolabelled ligand from the hERG channel. This assay is high throughput and low cost. It is suitable for primary screening to identify compounds that have a high affinity for hERG channel. Cerep uses both automated and conventional patch clamp to perform functional

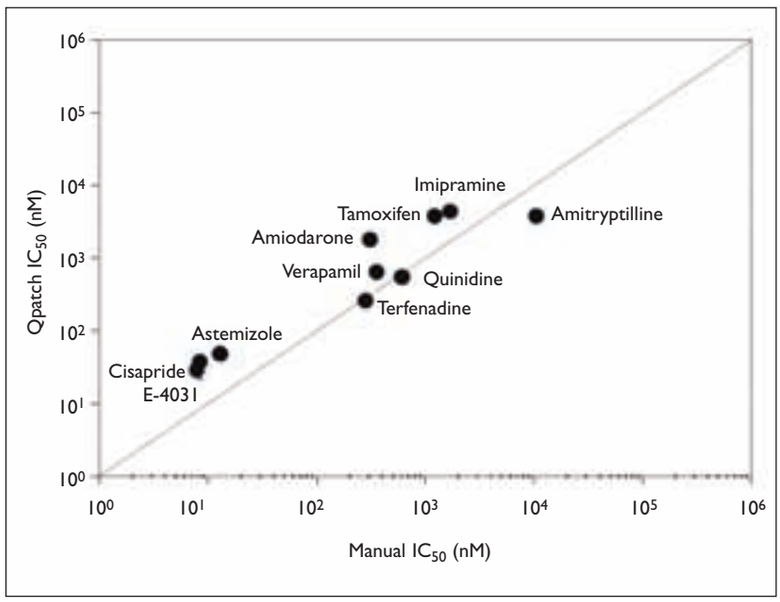


**Figure 17:** Comparison of $IC_{50}$ values of 10 reference compound determined by automated patch clamp (Qpatch) and conventional (Manual) patch clamp at Cerep

hERG liability study. Its automated patch clamp assay uses Qpatch 16 (Sophion Biosciences). It provides higher throughput, lower cost and comparable results with conventional patch clamp (**Figure 17**). Cerep offers hERG channel assessment by conventional patch clamp, which is the gold standard, for thorough study of the hERG liability. The test compound can be evaluated at a single or multiple concentrations (up to five). Customised protocols are also available. As an added value, the test concentrations can be verified by dosing solution analysis (optional) performed at Cerep. To meet the clients' need for regulatory submission of hERG assessment, Cerep offers GLP-compliant hERG conventional patch clamp assay. The GLP study is designed according to ICH-S7B recommendation and is fully compliant with the FDA GLP regulation. In addition to hERG liability assays, Cerep also develops and offers assessment of other ion channels, such as $Kv_{1.5}$, $Nav_{1.5}$ and so on, that contribute to the cardiac action potential.

| Available Now | | Available 2008 | |
|---|---|---|---|
| S49T3A | nAChR a 1 (CCL-136 cells) | ASIC-1 | P2X7 |
| BK | NaChR a 3 (IMR-32 cells) | Cav1.1 (Skm L-type) | SK1 |
| Cav1.2 (L-type) | Nav1.1 | CLC1 | SK2 |
| Cav2.1 (P/Q type) | Nav1.2 | CLC2 | TRPM2 |
| Cav2.2 (N-type) | Nav1.3 | CLC-K/Abartin | TRPP1/TRPP2 |
| Cav3.2 (T-type) | Nav1.4 | CLC-K/Babartin | |
| CFTR | Nav1.5 | CNGA1/CNGB1 | |
| EAG1 | Nav1.6 | CNGA3/CNGB3 | |
| HCN2 | Nav1.7 | ELK2 | |
| HCN4 | Nav1.8 | GABA-A receptor | |
| HERG1 | NCX1 | HCN1 | |
| KCNQ2/KCNQ3 | NMDA receptor (rat neuron) | HCN3 | |
| KCNQ3/KCNQ5 | NMDA receptor (NR1/NR2A) | IK | |
| Kir2.1 | NMDA receptor (NR1/NR2B) | KCNQ4 | |
| Kir3.1/Kir3.4 | P2X1 | Kir1.1 | |
| Kir6.2/SUR1 | P2x3 | Kir3.1/Kir3.2 | |
| Kir6.2/SUR2A | SK3 | Kir6.1/SUR2B | |
| Kv1.1 | TRPA1 | Kir6.2/SUR2B | |
| Kv1.2 | TRPC1 | Kv4.1 | |
| Kv1.3 | TRPC4 | NbAChR a 7 | |
| Kv1.4 | TRPC6 | Nav1.9 | |
| Kv1.5 | TRPM4 | P2X2 | |
| Kv2.1/Kv9.3 | TRPM6 | P2X4 | |
| Kv3.4 | TRPV1 | | |
| Kv4.2/KChIP2.2 | TRPV4 | | |

**Figure 18:** 'Books' available in ChanTest's Ion Channel Library, and those available by the end of 2008

**ChanTest** (www.chantest.com) is a rapidly growing discovery and safety services company specialising in ion channels. ChanTest is assembling, validating and optimising the world's largest library of ion channel-expressing cell lines for functional screening using automated patch clamp (MDS-AT PatchXpress® [4], IW Quattro™ [1] and Sophion QPatch [1]), fluorescence imaging plate readers ($FLIPR^{TETRA}$® and FLIPR 96) and a proprietary cell-based ELISA to measure ion channel surface expression (HERG-Lite®, CHAN-Lite™). 'Books' in the Ion Channel Library include ligand-gated, voltage-gated and mechanosensitive ion channels. The 'books' can be arranged as Channel Panels according to tissue (Cardiac Channel Panel™, Respiratory Channel Panel™), therapeutic area (Pain Channel Panel™, Epilepsy Channel Panel™) or ion channel family (Nav1.x, TRPx). The Channel Panels can be used for selectivity profiling when ion channels are primary drug targets or as off-target targets for safety and toxicology. Customers can also arrange their own Channel Panels for their specific needs. At present the library has 64 'books' (**Figure 18**), which will increase to 102 by the end of 2008 and 120 by mid-2009. HTS at 10,000 data points/day is achieved with automated fluorescence and ELISA methods, M(medium)TS with IW Quattro™ (2000 data points (DP)/day) and High Content Analysis with PatchXpress® (100DP/day) and QPatch (300DP/day). GLP patch clamp studies including the hERG assay use the standard reference manual patch clamp method. The ChanTest platform covers the entire preclinical ion channel spectrum from hits to leads, lead selection and optimisation and GLP IND applications. Evidence of ChanTest's expertise and competence in ion channels is demonstrated by the fact that it has done more than 1,000 GLP HERG assays for more than 200 customers and has been successfully audited by the FDA. Moving forward, Sophion Biosciences will provide ion channel cell lines from ChanTest that are optimised for Sophion's QPatch automated patch clamp systems.

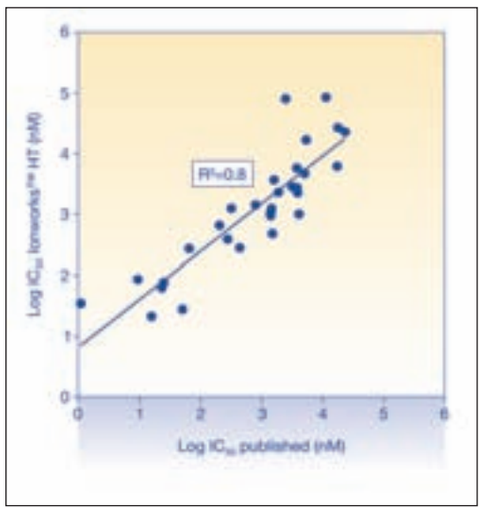


**Figure 19:** Comparison of Cyprotex hERG safety data generated using the Ionworks™ HT with published traditional patch clamp data (32 references available on request from Cyprotex)

**Cyprotex** (www.cyprotex.com) is a provider of both experimental and predictive ADMET and pharmacokinetic services. In 2005, it launched its Cloe® Screen hERG safety assay. This service is performed using the popular Ionworks™ HT platform (Molecular Devices using a CHO-hERG cell line) which measures whole-cell current from multiple cells simultaneously using an automated patch clamp system. Data generated in the Cloe® Screen hERG safety assay (Ionworks™ HT) compares well against published traditional patch clamp data ($R^2$ = 0.8) as illustrated in **Figure 19**. At Cyprotex, our systems are highly automated both in terms of liquid handling and data analysis. We have customised our own internal software for results calculation, system checks and results extraction. This ensures rapid, consistent high quality data. The efficiency of the automated patch clamp technique warrants it suitable for early stage screening and enables large banks of reliable data to be generated which are appropriate for building predictive models.

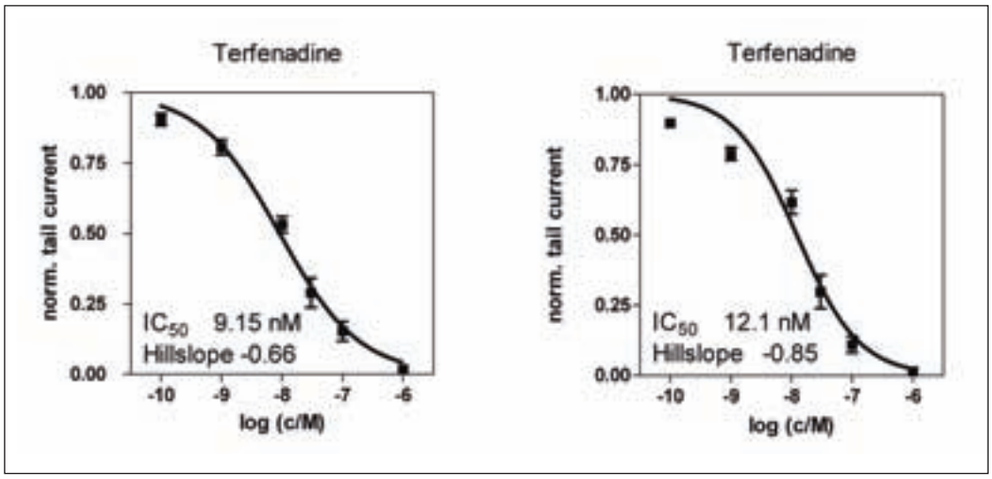


**Figure 20**
Concentration-response relationships using hERG-HEK293 CytoPAQ Instant cells obtained with the CytoPatch™ instrument (left figure) and conventional patch clamp (right figure). Data were generated from at least three different cells. The data indicate that the CytoPatch™ Instrument shows the same data quality as a conventional patch clamp workstation

**Cytocentrics** (www.cytocentrics.com) provides innovative solutions in the area of ion channel screening with a main focus on patch clamp: CytoPatch™, an in-house developed patch clamp instrument; ready-to-patch CytoPAQ Instant Cells; and Ion Channel Screening Service. As a CRO, Cytocentrics has the claim to offer a premium service to its customers. Indispensable for the generation of reliable and reproducible patch clamp data is an excellent cell quality. Even slight

fluctuations in cell treatment may result in profound changes in membrane stability and ionic current amplitudes. Therefore, Cytocentrics developed the CytoPAQ Instant Cells. Every batch of these cells is validated by Cytocentrics' experienced electrophysiologists in regards to PAQ = 'Performance, Accuracy and Quality'. The hERG-HEK CytoPAQ Instant cells possess a constant and equal high cell quality and are used routinely by Cytocentrics for safety screening service. A portfolio extension of the CytoPAQ Instant cells expressing further important ion channels is in development and will enter the market at the beginning of 2009. The electrophysiological platform consists of conventional patch clamp workstations optional with slow or fast perfusion (Dynaflow® System) and several CytoPatch™ Instruments. All services are performed using the CytoPAQ Instant Cells and will be offered as GLP and non-GLP study on both patch clamp platforms. Cytocentrics also conducts hERG channel trafficking assays to assess the potential of compounds to inhibit hERG trafficking (**Figure 20**).

At **Evotec** (www.evotec.com) several teams of experts collaborate to support ion channel projects. A team of molecular biologists have expressed more than 30 ion channels and these are accessible for screening or profiling. The screens are performed using a proprietary high throughput technology based on voltage-sensitive dyes, in addition to calcium-sensitive dyes. In several projects the use of diverse compound libraries are found beneficial, as chemical libraries selected on known ion channel pharmacophores frequently result in unselective hit compounds. The screens were performed back-to-back with selectivity screens on related channels to progress not just with the most active, but also selective compounds. At later stages the compounds are evaluated using electrophysiology, the 'gold standard technology' in this field. In the past, this stage was seen as a bottleneck. However, new automated patch-clamp technologies now allow sufficient throughput to validate compounds, allow testing of molecules arising from the medicinal chemistry and in some instances primary screening. Medicinal chemists then use *in silico* methods to prioritise molecules. The collaboration of experts in each key area results in the effective and efficient support for ion channel projects, not only for its partners in the pharma and biotech industry, but also for its internal drug discovery projects (**Figure 21**).

**Figure 21:** Manual patch-clamping – one of a whole array of capabilities offered by Evotec for ion channel testing

**IonGate** (www.iongate.de) offers screening services using SURFE$^2$R Technology, its proprietary technology platform. The sensor-based system uses membrane preparations from cultured cells or tissue samples as well as liposomes or intracellular membrane preparations to investigate the function of transporters and anion channels. Activity is measured via charging currents of membrane fragments or liposomes which are capacitively coupled to the sensor surface. Available services range from detailed analysis of biophysical properties via investigation of pharmacological parameters to screening of focused compound libraries. Using membranes directly prepared from tissue samples, it becomes possible to perform studies on proteins that were expressed in the target organ under *in vivo* conditions, making the results more predictive than those from conventional cell culture. Examples for proteins investigated for specificity testing include Na/K-ATPase (kidney, synaptosomal membranes and heart), NCX (heart), H/K-ATPase (stomach), V-ATPase and Cl channels (synaptic vesicles) and Ca-ATPase (muscle). IonGate has extensive experience in conducting customer projects. More than 100 customer projects were completed successfully. Apart from SURFE$^2$R experiments IonGate can carry out all necessary standard procedures starting with molecular biology, cell culture via membrane preparations to assay development. Customer projects can therefore seamlessly be taken over at any point of project progress.

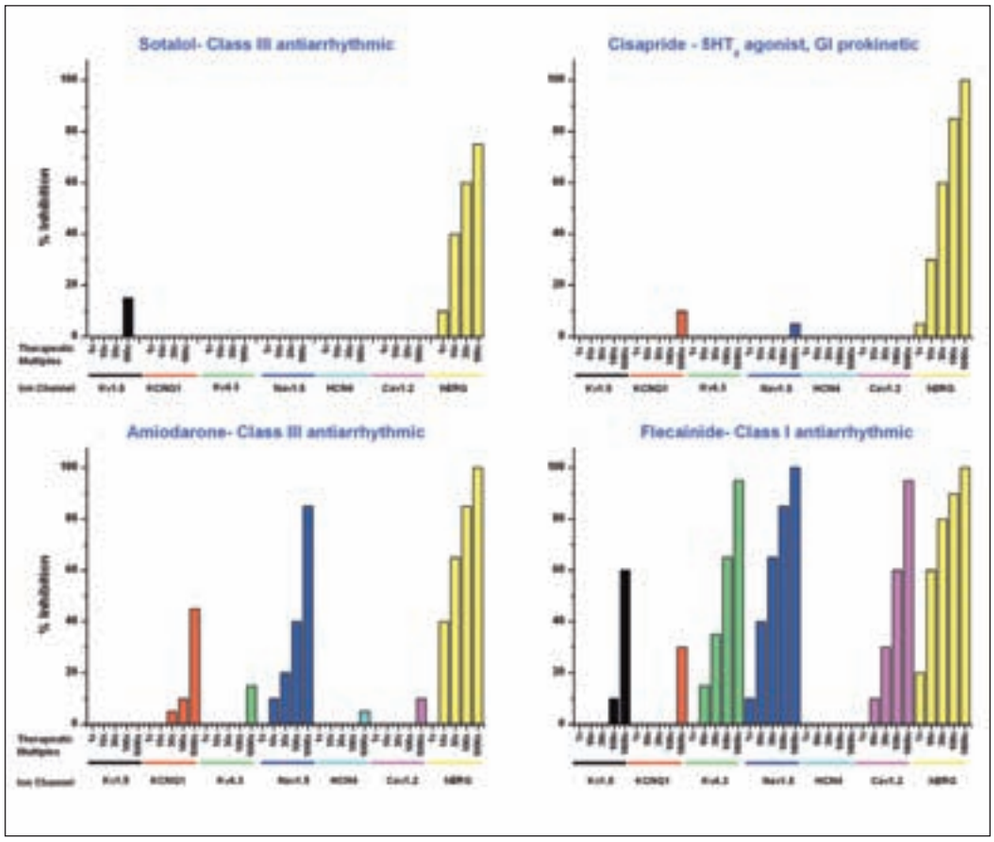


**Figure 22**
Millipore's CardiacProfiler panel has been benchmarked using reference compounds with known *in vivo* activities and therapeutic concentrations. The cardiac cell lines tested are indicated under the relevant coloured bar. Note that KCNQ1, $Kv_{4.3}$ and $Cav_{1.2}$ are multi-subunit channels since the pore-forming unit is co-expressed with minK, KChIP1 and β2a + α2δ1 accessory subunits respectively

**MDS Pharma Services** offers more than 62 ion channel testing assays (http://discovery.mdsps.com/Catalog/Discovery/LeadSelection/IonChannel/Intro.aspx), encompassing receptor binding, functional cellular patch clamp, tissue and *in vivo* assays to fully characterise compound interaction with ion channels for potential safety considerations such as those seen with the torsade de pointes (TdP) and other ion channel interaction side-effects, or for target identification. Our ion channel testing services cover three families of ion channels: 1) Voltage-gated channels: The voltage-gated channel family includes $Na^+$, $Ca^+$ and $K^+$ channels whose open and closed states are mediated by changes in cell membrane potential. These multimeric channels tightly regulate the flux of ions across cell membranes and maintain ionic gradients required for normal cell function including but not limited to, neurons, skeletal and cardiac muscle cells; 2) Transient Receptor Potential channels: The TRP family contains more than 28 members with diverse modes of activation. TRP subfamilies include classical (TRPC), vanilloid receptors (TRPV), melastatin (TRPM), polycystins (TRPP), mucolipins (TRPML), and ankyrin transmembrane protein 1 (TRPA). Some TRP channels are voltage-gated, though not strongly so, with others sensitive to intracellular $Ca^{2+}$, pH, redox state, osmolarity, and mechanical stretch; 3) Ligand-gated channels (LGICs): The LGICs open in response to specific ligand binding to the extracellular domain of the receptor. Ligand binding induces a conformational change causing the channel gate to open and allow ion flux across the plasma membrane. LGICs include the Nicotinic Acetylcholine receptor, ionotropic glutamate receptors, P2X receptors, and $GABA_A$ receptor.

**Millipore**'s (www.millipore.com) capabilities include a range of electrophysiology assays and services that are designed to support and accelerate all stages of ion channel drug discovery. These IonChannelProfiler™ services are aimed at a variety of sizes and types of project, from HTS screening or SAR against individual targets to profiling smaller sets against selectivity or liability panels or detailed mechanistic studies of single compounds. Based on 'purpose-built' in-house cell lines, the assays use two types of automated platform (IonWorks and PatchXpress) as well as manual patch clamp. Standardised protocols have been developed and are

optimised, for example to detect state-dependent blockers, using known pharmacological agents as references. Protocols can also be customised as part of the bespoke 'FlexLab' service. The portfolio includes a wide selection of voltage-gated channels as well as an increasing range of ligand-gated channels, the latter harnessing recent advancements in automated electrophysiology. New assays and cell lines are continually being added and custom target development capabilities are included within the 'FlexLab' service. Millipore also provides cardiac safety profiling services including functional hERG screening at differing throughputs and levels of detail using Ionworks, PatchXpress and manual patch clamp. Validated hERG membranes are also available for conventional ligand binding studies. Millipore's CardiacProfiler™ panel includes eight key channels with important roles in myocyte action potential generation and/or inherited long QT syndrome. This comprehensive panel is run as a monthly service offering a cost-effective way of screening single compounds or larger compound sets against these key liability channels (**Figure 22**).

## Summary

It is very clear from HTStec's report that there is significant demand today in Pharma and Biotech, and also in some academic groups, for accessing ion channel testing services and this is reflected by the number and the variety of service offerings. In addition, it was evident in the survey and from the vendor updates that APC systems are undertaking an ever increasing role in the services that are offered and a considerable wealth of experience has accrued in their use. The survey found that the majority of GLP cardiac safety hERG testing is primarily outsourced today, while non-GLP is frequently done early in-house. Although manual patch clamping is still regarded as the 'gold standard technology' for GLP cardiac safety hERG testing, it is apparent that APC systems are now being seriously considered as alternatives in this respect, with many providers offering both manual patch and APC hERG channel services in parallel. In addition, several vendors have pointed out in this review good correlations exist between manual patch and APC systems for the hERG channel and data quality is not sacrificed with APC-derived testing. Overall this view is reflected in the technology preferences of HTStec's survey respondents, with more than 50% agreeing that alternatives to manual patch clamp should be considered for GLP testing. In the highly competitive area of cardiac safety hERG testing what now appears to be driving selection of a CRO is the price of the service and the turn-around time. While for outsourcing ion channel drug discovery (primary screening and selectivity profiling) data quality, access to ion channel targets not currently available in-house and the technology (ie the methodology used to test compounds) are of greater or equal importance.

CROs are also competing to offer new services, this is particularly the case for ion channel expressing cell lines. Many now offer rapid cell line development with high target expression, suitable for fluorescence and patch-clamp assays and are stressing the reliability and quality of their validated assay-ready cells. Few organisations have sufficient resources to generate and maintain the breadth of different cell lines that are needed to support diverse profiling panels and this is an obvious area where CROs can exploit economies of scale and have recently begun to aggressively expand their capabilities. Several companies are assembling very large ion channel libraries, variously grouped in panels (eg by tissue, therapeutic area and/or ion channel family), for use in selectivity profiling where ion channels are the primary drug targets or are off-targets for safety and toxicology.

Other services on the increase include the capability to accept larger libraries for primary screening and various cardiotoxicity assessment offerings. The number of CROs providing medicinal chemistry support, ion channel directed chemical libraries and even computational models to forecast hERG inhibition looks set to grow. For the future, screening services involving primary cells, cardiomyocytes and even stem cells differentiated *in vitro* into various cell lineages such as neurons, for testing to get even closer to the *in vivo* state of the channel is envisaged.

In conclusion current interest in outsourcing ion channel services is high and looks set to be a growth area in the future. With the large number of vendors vying for business, customers can expect competitive pricing and good turn-around times. DDW

***Dr John Comley** is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery. Since it formation in 2003, HTStec has published 35 market reports on drug discovery technologies and Dr Comley has authored 24 review articles in* **Drug Discovery World**. *Further information on accessing the market report 'Ion Channel Trends 2007' can be obtained by visiting www.htstec.com or by emailing john.comley@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*

## Reference

1 Ion Channel Trends 2007 Report, published by HTStec Limited, Cambridge, UK, June 2007.